The influence of mechanical resistance on the development and life-period of cells.

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Introduction.

The question as to what actively growing plant tissues will do when their growth is checked by external mechanical resistance had received but small notice in literature till the appearance of Pfeffer's[1] latest published work. Some years ago, however, De Vries,[2] by winding stems with twine, found that the cambium gave rise to fewer cells under this resistance and that the wood elements expanded more slowly. Krabbe[3] by applying a graduated pressure to the trunks of trees, found the cambium cells uninfluenced either in size of lumen or in thickness of wall both when the cambium was forming new cells under various pressures and when the formation of new cells was entirely stopped by sufficiently increasing the resistance. This author confirmed De Vries' observation stated above, that the time between the formation of a wood element and its definitive condition was lengthened by increasing the pressure under which it grew. Wortmann[4] placed bandages of twine about the stems of seedlings of Phaseolus multiflorus and two other seedlings and found within a few days that the subepidermal collenchyma was abnormally thickened. This thickening he used to strengthen his theory of growth, believing that cell-walls grew thicker than normally when they could not reach their normal surface extension.

Pfeffer in the work referred to enclosed the root-tips of several species of seedlings, the stems of a few species, the growing points of Chara and Nitella, and the filaments of Spirogyra in gypsum casts. From the behavior of these preparations he deduces these results:

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[1]Pfeffer, Druck und Arbeitsleistung. Abhandlungen der könig. säch. Gesells. der Wissenschaft **20:** —. 1893.

[2]De Vries, De l'influence de la pression du liber sur la structure des couches ligneuses annuelles. Extrait des Archives Neerlandaises 1876. Also, Vorläufige Mittheilung, Flora —: 97–102. 1875.

[3]Krabbe, Ueber das Wachsthum des Verdickungsringes und der jungen Holzzellen in seiner Abhängigkeit von Druckwirkungen. Berlin, 1884.

[4]Wortmann, Beiträge zur Physiologie des Wachsthums. Bot. Zeit. **47:** 286. 1889.

1. Embryonal tissue preserves for a long period in a gypsum cast its capability for growth.

2. In gypsum casts the differentiation of tissue advances nearer the growing point than normally, thus necessarily shortening the zone capable of elongation.

3. The cells of the embryonal tissue do not divide when their extension is prevented, but the cells of the tissue adjacent to the embryonal tissue do, in some plants at least, divide at a size somewhat less than their normal.

The following pages contain further observations on the questions already stated and besides show the effect of external mechanical resistance on

1. The duration of the growing period of cells;
2. The duration of the life period of cells;
3. The permanent condition assumed by cells.

This work was begun in Leipzig under the direction of Professor Pfeffer and completed in Michigan University since the author's return.

Methods.

The method used to arrest growth by mechanical means has been the employment of gypsum casts.[5] An organ to be encased had fitted about it an envelope of stiff paper closed at the bottom by a divided cork, by molding clay, or by cotton wool. A thick mixture of gypsum and water was stirred up and poured into the envelope and there allowed to harden. Two precautions are necessary to the securing of good results: the cast must have a diameter several to many times that of the organ encased to prevent springing by the energy of turgor;[6] the cast must have a length of three or more centimeters, since experience has shown that disturbing factors come into play in proximity to the limits of the gypsum envelope.

At the close of the experiment, the preparation was cut from the plant, the enclosed organ removed from the cast and subjected to microscopical examination. To free the organ from the gypsum, two longitudinal trenches were cut with a

[5] Pfeffer has used this method in researches for some time and has described it in *Berichte d. k. saechs. Gesellsch. d. Wiss.*, Dec. 1892: Ueber Anwendung des Gipsverbandes für pflanzen-physiologische Studien.

[6] Krabbe found the outward pressure of turgor under a ligature to reach fifteen atmospheres in the stems of dicotyledonous trees (l. c.), and Pfeffer found the pressure given by the roots of seedlings in gypsum casts to reach in some cases twelve atmospheres, while in the stems of some seedlings the pressure was six and one-half atmospheres. (Druck u. Arbeitsleistung, p. 188.)

knife or saw from opposite sides of the cast down nearly to the plant organ enclosed. The halves were then easily broken apart without injury to the plant tissue.

The cells were regarded as living or dead according as plasmolysis was present or absent after placing sections in a ten per cent. solution of potassium nitrate.

The following plants were used in the experimentation:

Allium cepa L.,
Althæa tauriensis DC.,
Archangelica sativa Mill.,
Caltha palustris L.,
Cucurbita pepo L.,
Dahlia variabilis W.,
Equisetum limosum L.,
Eryngium planum L.,
Forsythia viridissima Lindb.,
Helianthus tuberosus L.,
Juglans nigra L.,
Juncus effusus L.,
Lamium garganicum L.,
Ligusticum Leguari,
Melianthus major L.,
Myrrhis odorata Scop.,
Phaseolus multiflorus Lam.,
Phytolacca dioica L.,
Pterocarya fraxinifolia Nutt.,
Ricinus communis L.,
Sambucus nigra L.,
Triticum repens L.,
Urtica dioica L.,
Vicia faba L.,
Zea mais L.

Experiments and discussion.

Effect of mechanical resistance on the growth and preservation of meristematic tissue.

In operating upon the growing points of the roots and stems of several species of seedlings, Pfeffer[7] found that within a gypsum cast which prevented all extension of tissue, the ability for growth was retained for many weeks. In the cases tested, growth was immediately renewed on removal of the cast. Here, then, with all the conditions of growth favorable, except the space in which to extend, the primary meristem retains its functional capability as it does when obliged to rest by low temperature or by insufficient moisture. Pfeffer found also the growing tips of Chara and Nitella living after being three months in gypsum.

My own experiments have shown that intercalary meristem and cambium as well as growing points retain their functional capability for long periods when their growth is prevented by similar gypsum casts. In Juncus, as is well known, the meristem for the growth of the aerial stems is at the place where such stems grow off from the rhizome. Casts were placed around the bases of many stems and about the rhizomes so as to include the zone of meristem. Some of these preparations were examined five weeks afterward, some after eleven weeks.

[7] Pfeffer: l. c., p. 124.

The cells of the meristematic zone were in all cases living and normal in appearance at both periods of examination.

The growth of the leaves of Allium cepa is also intercalary; the meristem is at the leaf-bases. Several experiments were made on this plant by including within a cast the upper part of the bulb and the young leaves which had started from it. The cast was then fastened by bandages to the bulb so that bulb and cast could not separate, yet so that the roots could grow out freely. The preparations were examined, some at the end of two weeks, some after thirty-one days. The results were the same in all cases. The meristem remained alive, and growth was resumed upon removal of the casts.

The effect on the cambium of arresting its growth by external resistance has been determined by enclosing within gypsum casts the stems of many plants both herbaceous and woody. To prevent by this method all extension or growth in the cells within is impossible, since the presence of intercellular spaces always affords some room, and the resistance of vessels is not sufficient to withstand the force of turgor of the thin-walled cells. Thus, though the cambium has not been held in these experiments, and could not be, in a state of absolute rest as regards growth, its activity in this direction has been, as will be seen, very slight. The amount of growth from the cambium in such circumstances must depend entirely on the room it can make for itself, and differs therefore in different species of plants, and differs also in the same species, since the size of the intercellular spaces and the turgor of a tissue will differ with age. In young stems of many plants the primary meristem of the fibrovascular bundles has been preserved in an almost quiescent condition for several weeks. Rarer examples of long continued rest have been furnished by Lamium garganicum, Vicia faba and Dahlia variabilis in which so slight was the development within the casts that for 40, 50 and 120 days respectively the formation of the interfascicular cambium was prevented, though it was formed immediately below and above the limits of the casts, and the growth of the plants as a whole continued. Yet notwithstanding this long rest the primary meristem was apparently and undoubtedly capable of farther active growth.

In cases where the casts were applied after the cambium zone was completed, there are many individuals, representing several species, bearing testimony to the long preservation

of this meristematic tissue when its growth is mechanically checked. In none of these cases were the experiments continued till the death of the cambium, and hence the duration of its vitality, when its growth is mechanically prevented, is still undetermined. Pfeffer, in the work mentioned, states that the root-tips of Vicia faba in casts remained alive for five weeks, but that at the end of ten weeks had begun to die. The cambium certainly lives longer under similar conditions. Cucurbita pepo with considerable growth of stem outside the cast and very few changes within the cast has preserved its cambium for sixty-six days. Eryngium planum and Ligusticum Leguari about whose stems casts were placed at the time the cambium ring was completed, grew well afterward, forming outside the casts normally thick stems, and at the time the plants were taken for examination had produced seeds; the cambium was thus preserved in these species for seventy days. Young plants of Vicia faba grew after the second or third epicotyledonary internode had been encased in gypsum to a size and development equalling normal plants and had seeds partially formed when the plants were cut for examination 116 days after placing in gypsum. Dahlia variabilis does not grow very well when a cast is put around a very young stem. Several individuals however added half a meter to their height and were still growing and had healthy looking cambium within the cast 138 days after the beginning of the experiment. Pterocarya fraxinifolia, Juglans nigra and Forsythia viridissima formed branches in most cases as well developed as normal ones, though the same branches when young had had gypsum laid around them, and within the cast formed but few seconday elements, Forsythia in one case forming but four or five in a radial row. This experiment was continued for seventy days, at the end of which period the cambium appeared normal. In similar conditions and with similar results Sambucus nigra was grown for ninety-six days, Ricinus communis for 100 days and Phytolacca dioica for 197 days. In the last named plant, in one stem, there had been formed within the cast five or six cambial derivatives in a radial row after the experiment was begun, while above and below the cast thirty to thirty-five such cells had been formed.

It is to be understood that in all these cases the stems had increased greatly in diameter beyond that of the part within

the cast; as extreme examples may be mentioned Vicia, Dahlia, Ricinus and Pterocarya where the diameter outside the cast was often to that within as two to one.

Effect of mechanical resistance on the duration of the period of development of cells.

1. *On the zone of elongation in roots and stems.*—When root-tips or stem-tips of seedlings are fixed in a gypsum cast, the power of elongation becomes day by day reduced to narrower limits, so that when the growing point is released from its confining envelope, subsequent growth shows that the proximal limit of elongation is nearer the apex of the organ than formerly. Pfeffer[8] demonstrated this in several species. In the primary root of Vicia faba, for instance, where normally the elongating zone is about 10^{mm}, he found this zone reduced to 5^{mm} or 6^{mm} after two or three days in a cast. My own measurements have shown that in a normally growing primary root of Vicia faba at a temperature of 20°, the fourth millimeter from the apex of the root will in twelve hours have passed out of the segment of elongation. But Pfeffer's root-tips showed elongation in the fifth or sixth millimeter after two or three days in casts. Thus it is evident that the effect of the casts was to retard the passage of the elongating segment into permanent tissue.

Analogous with this result is that obtained in my experiments with Juncus and Lamium, where several very short shoots of the former in which tissues were undifferentiated were kept alive for eleven weeks in casts, and then showed no differentiation; and in the latter the stem just behind the terminal bud was, in one case for twenty-five days, in another for forty-five days, by the same means kept from developing farther, except that two or three cells in the primary bundles slightly thickened their membranes. Meanwhile the stems had grown above the casts and the tissues had become much better developed than within the casts.

2. *On differentiation in fundamental parenchyma.*—Not only in the tissue adjoining the meristem of growing points will development proceed more slowly when a mechanical resistance prevents expansion, but in those later changes which in many plants the fundamental tissue undergoes will the same result follow. In Zea mais the cells which normally form the

[8] Pfeffer, l. c., pp. 120 and 149.

sclerenchyma sheaths have, by the employment of casts around the stem, been kept thin-walled for thirty-seven days, in Caltha palustris for fifty-two days, though these cells remained alive and in the same stems above and below the casts passed into their thick-walled condition.

Other plants in which the outer zone of pith-cells normally becomes thick-walled have served still better to illustrate this principle. Numerous examples of Vicia faba have shown that the outer pith-cells begin to thicken their membranes two or three weeks after their internode is fully elongated. If, however, a cast is laid around a very young internode, the thickening of the membranes of the pith-cells will be delayed for weeks after it has begun in the internodes of the same stem above and below the cast. Thus in a stem that had grown to the height of ten internodes, whose third internode above the cotyledons had been encased in gypsum before elongation was complete, the subsequent period of growth being thirty-two days, the pith-cells beyond the limits of the cast were becoming thick-walled, while within the cast they retained their thin-walled condition. Other plants of the same species similarly treated but allowed to grow twelve days longer, at which time they had added to their height and begun to blossom, showed within the limits of the casts the outer pith-cells just beginning to thicken their membranes. Similar preparations, but twelve days older, and consequently of stronger development, showed the outer pith-cells with membranes thickened, but still thinner than in normal parts of the stems. Urtica dioica, twenty-three days after two or three young internodes were enclosed in a cast, had above the casts thick-walled pith-cells bounding the inner ends of the bundles, but only thin-walled cells in similar positions within the casts. Other stems growing for forty days after casts were applied in like manner, showed within the casts the outer pith-cells with thickening membranes. In Dahlia variabilis the outer cells of the pith have by means of casts been kept thin-walled ten weeks later than in neighboring normal parts of the same stems. Still older preparations have shown however that if growth in the plant as a whole continues these cells will eventually become thick-walled. Archangelica sativa and Myrrhis odorata form a broad zone of mechanical pith which bounds primary and secondary xylem internally. Segments of stems of these two species have presented within casts the

pith entirely thin-walled, when above and below the casts the zone of fully thickened pith-cells has been six cells in radial width.

If we turn now to woody plants we shall find the same results presenting themselves. Several shoots of Melianthus major had casts placed around them so as to leave only the terminal bud exposed above. Up to the time when three internodes had been subsequently developed there were no thick-walled cells in the pith of the segment in cast, but above and below the cast there was a broad band of thick-walled lignified pith. In shoots similarly prepared but of farther development above the casts, there was always an evident thickening of the outer pith-cells within the limits of the casts, this thickening progressing in the older preparations till it approached that of like cells outside the confined area. The same general results were obtained by similar experiments with Forsythia viridissima and Pterocarya fraxinifolia.

Many plants which have collenchyma in the cortex of the young stem do not, as is well known, increase the amount of this tissue as growth proceeds, while others with increasing age in an internode show the collenchyma increasing in number of cells and thickness of membrane. Sambucus nigra belongs to the latter class. When in the spring very young shoots have some of their internodes enclosed in gypsum and are allowed to grow subsequently, the increase of the collenchyma is found to be more tardy within the casts than outside of them, though the thickening of cell-walls is still slowly progressive within the casts.

In the young stems of Archangelica sativa and Myrrhis odorata the collenchyma strands of the future exist in a very thin-walled condition. By laying gypsum around segments of such young stems, the strands referred to have been in Archangelica for twenty days, in Myrrhis for twenty-seven days, kept in their thin-walled condition, while above and below the limits of the casts the strands in the same time formed very thick-walled collenchyma. How much longer these cells would have remained thin-walled within the cast was not determined by other experiments; but that they were still capable of growth there can be no doubt, for they were at the time of examination well provided with protoplasm.

The fact that the sclerenchyma of the fundamental tissue develops more slowly against mechanical resistance has been

mentioned for Caltha palustris and Zea mais. The same is true of the only other plant in which the question was studied, viz., Cucurbita pepo. In this plant after elongation of the internodes the innermost cortical cells thicken into a heavy zone of sclerenchyma. In internodes confined in casts this zone has been delayed in development into sclerenchyma for several weeks, though it could there be identified as a band of thin-walled cells. Older preparations have shown this band becoming slowly thick-walled, the progress continuing after the full thickness of wall had been obtained in parts above and below the cast; though in the oldest preparation examined these cells had not become so thick as in normal parts of the same stem.

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[*To be continued.*]